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ORIGINAL ARTICLE

Effects of sphingosine-1-phosphate on gene expression of two cell mouse embryos induced by C_2-Ceramide

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KEYWORDS

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Abstract *Objective:* As to understand the sphingosine-1-phosphate (S1P) on gene expression of the pre-implantation mouse embryo induced by C_2-Ceramide (CED).

Materials and methods: Global gene-expression profiling of two cell mouse embryo was performed using Affymetrix Gene Chip® Mouse Genome 430 2.0 Array.

Results: Fifty-five genes were identified with significant expression changes that are functionally involved in the two cell embryo after treated by S1P and CED. Of these genes, 30 were up-regulated and 25 were down-regulated. Genes *Atm*, *Cdkn1b* and *Fgrfr2* are identified to be involved in the protective role of S1P against the apoptotic signals induced by CED.

Abbreviations: AC, acid ceramidase;Atm, ataxia-telangiectasia-mutated; CED, C_2-Ceramide; Cer, ceramide; CDKs, cyclin-dependent kinases; Cdkn1b, p27, cyclin-dependent kinase inhibitor 1B; FGF, fibroblast growth factor receptor;GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; MAPK, mitogen-activated protein kinase; Sph, sphingosine; S1P, sphingosine-1-phosphate; PIKK, phosphoinositide-3-kinase-related protein kinases.

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Conclusions: This study provides a map of genes in the pre-implantation two cell mouse embryo. Further investigation based on these data will provide a better understanding of the effects of S1P on the pre-implantation embryos in other mammalian species, especially human.

1. Introduction

Sphingolipids are present in all eukaryotic cells where they contribute to membrane biology and signaling events which influence cell behavior and function (1). Sphingolipid metabolites ceramide (Cer), sphingosine (Sph) and sphingosine-1-phosphate (S1P) play important roles in the regulation of cell proliferation, survival and cell death. Cer and Sph usually inhibit proliferation and promote apoptosis, while S1P stimulates growth and suppresses apoptosis. Therefore, the cellular balance of these sphingolipid metabolites, the ''sphingolipid rheostat'', is of crucial importance in regulating cell fate (2). S1P is utilized as a regulator of cell function not only by binding to extra cellular receptors but also work as an intracellular second messenger (3). Cellular levels of S1P are regulated by the activation of the enzymes that are responsible for its synthesis and degradation. In mammalian cells, two sphingosine kinases, SphK1 and SphK2, catalyze the phosphorylation of sphingosine to generate S1P (4). SphK1 is generally regarded as providing pro-survival signals, whereas SphK2, at least when overexpressed, induces apoptosis in several cell types (5).

Additionally, ceramide is the central core in sphingolipid metabolism, but has also been involved in the regulation of signal transduction processes. Specifically, ceramides can induce cell cycle arrest and promote apoptosis, a form of programmed cell death (6,7). Cell ceramides typically have long N-acyl chains ranging from 16 to 26 carbons in length (8,9). However, in this study short-chain analog C_2-Ceramide has been used, because this is more water soluble than long-chain ceramides.

S1P is also known as an anti-apoptosis substance that acts via the suppression of ceramide (10,11). Several studies show that S1P protects the somatic cells (12), ovary tissues (13), oocytes (14–17), male germ cells (18,19) and embryos (16,20,21) from apoptosis by radiation and heat shock, and so on. Recently, addition of S1P to human embryo culture medium can decrease embryo fragmentation (16). Thus, S1P research could hypothetically pave the way for novel research avenue for improving the clinical outcomes of human assisted reproductive technology. The aim of this study was to evaluate the effect of S1P on the development, apoptosis-related gene expression of pre-implantation two cell mouse embryos in vitro.

2. Materials and methods

2.1. Embryo collection and culture

CD-1 mice were randomly bred and used for experimentation with approval of the Southern Medical University Animal Ethics and Experimentation Committee. Females (4–6 weeks of age) were super-ovulated by i.p. injection of 5 IU pregnant mare serum gonadotropin (PMSG, Livzon, China) followed 48 h later by 5 IU human chorionic gonadotropin (HCG, Livzon, China) and paired individually with males. Mating was determined by the presence of a vaginal plug at the following morning. Zygotes were collected 20 h after injection with HCG and cumulus cells were removed by 0.1% hyaluronidase treatment (22,23).

Embryos were randomized into four groups: the blank group cultured in CZB (MR-019-D, Millipore), the CED group cultured in CZB and 50 uM Ceramide (CED, Sigma–Aldrich, MO, US), the CED + methanol group cultured in 50 uM ceramide and 25 nM methanol, and the CED + S1P group cultured in 50 uM ceramide and 25 nM sphingosine 1-phosphate (S1P, Sigma–Aldrich, MO, US). The embryos were cultured in CED with or without equal volume of methanol as the sham control. All embryos were cultured under liquid paraffin oil at 37 °C in a humidified atmosphere of 5% CO_2, 95% air.

2.2. Total RNA isolation for micro array analysis

By using RNeasy Micro Kit (74004, Qiagen), total RNA was extracted from 350 two cell embryos of each group according to the manufacturer's instructions: the blank group (CZB only), the CED group, the CED + methanol group and the CED + S1P group. RNA from each group of embryos was isolated, amplified, and hybridized to the Affymetrix chip (Affymetrix, Santa Clara, CA) at the same time to minimize experimental bias. Total RNA was dissolved in 10 uL of sterile water and stored at −80 °C. Ribonucleic acid mass and size distribution were determined using Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA).

2.3. Micro array experiment

The gene expression profile of each sample was examined using mouse genome oligonucleotide micro array (Affymetrix Gene Chip Mouse Genome 430 2.0 Array; Affymetrix, Santa Clara, CA, USA) at Capital biochip Corporation (Beijing, China) in which the gene chip micro array service is certificated by Affymetrix. The chip measures the expression of over 45,000 probe sets representing over 34,000 well-substantiated mouse genes. The protocol for micro array processing was carried out according to the Gene Chip® Expression Analysis Technical Manual (Affymetrix, 701021, Rev.5). The hybridized micro arrays were then scanned using the GeneChip™ Scanner 3000 and converted into TIFF images in the preparation for analysis.

Table 1 KEGG and genome pathways enriched in differentially expressed gene of two cell mouse embryos induced by CED.

Pathway name	Related gene	*p*-Value	*q*-Value
MAPK signaling pathway	25	1.47E-20	1.77E-18
Cell cycle	17	3.57E-17	1.38E-15
p53 signaling pathway	10	1.29E-10	1.18E-09
Oxidative phosphorylation	13	2.42E-10	1.98E-09
Apoptosis	10	1.48E-09	1.02E-08